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Evaluation of Canadian barley breeding lines for Fusarium head blight resistance

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He, X., Osman, M., Helm, J., Capettini, F. and Singh, P. K. 2015. **Evaluation of Canadian barley breeding lines for Fusarium head blight resistance.** Can. J. Plant Sci. **95**: 923–929. Fusarium head blight (FHB) is a major challenge to the successful production of barley in Canada, as well as for end-users such as the malting and brewing industries. Due to the quantitative inheritance of FHB resistance, continuous effort is required to identify breeding lines with improved FHB resistance and incorporate them into crossing schemes to enhance FHB resistance. In the present study, 402 advanced breeding lines from Alberta, Canada, were evaluated in the FHB screening nursery at CIMMYT, Mexico. In 2011 and 2012, FHB incidence was measured on a scale of 1 to 4 to eliminate the most susceptible lines. In 2013 and 2014, 181 lines with the lowest disease scores in the previous 2 yr were tested in replicated experiments for field FHB index, Fusarium-damaged kernels, and deoxynivalenol content. Agronomic and morphological traits, specifically days to heading, plant height, and row and hull types were also evaluated in relations to FHB parameters. Correlation coefficients among the three FHB parameters in both 2013 and 2014 were all significant at $P < 0.0001$, ranging from 0.36 to 0.63. Additional correlation analysis showed that late-maturing, tall, and two-row lines tended to have lower disease, whereas hull type did not show a significant correlation with FHB. Several lines with high and stable FHB resistance similar to that of the resistant checks were identified. These could be used in breeding programs as resistance sources or be registered as new cultivars if their overall attributes meet commercial standards.

Key words: Deoxynivalenol, Fusarium head blight screening, Fusarium-damaged kernels, *Fusarium graminearum*, *Hordeum vulgare* L.

He, X., Osman, M., Helm, J., Capettini, F. et Singh, P. K. 2015. **Évaluation de la résistance des lignées généalogiques canadiennes d'orge à la brûlure de l'épi causée par Fusarium.** Can. J. Plant Sci. **95**: 923–929. La brûlure de l'épi attribuable à Fusarium (FHB) est un obstacle majeur à une culture rentable de l'orge au Canada; cette maladie pose aussi un problème aux utilisateurs, notamment à l'industrie brassicole. La résistance à la FHB étant un caractère héréditaire quantitatif, on est constamment à la recherche de lignées généalogiques plus résistantes, dont les gènes seront intégrés à d'autres dans le cadre de programmes d'hybridation visant à accroître cette résistance. Les auteurs ont évalué 402 lignées généalogiques avancées de l'Alberta (Canada) à la pépinière de présélection du CIMMYT, au Mexique. En 2011 et 2012, l'incidence de la FHB a été mesurée sur une échelle de un à quatre, de manière à éliminer les lignées les plus sensibles. En 2013 et 2014, les 181 lignées les moins atteintes au cours de deux années antérieures ont été testées dans le cadre d'expériences répétées visant à établir l'indice de la FHB au champ, le nombre de grains abîmés par Fusarium et la concentration de désoxynivalénol. Les chercheurs ont également évalué les caractères agronomiques et morphologiques (nombre de jours avant l'épiaison, taille du plant, nombre de rangs et présence ou pas de glumes) en fonction des paramètres de la FHB. En 2013 et 2014, les coefficients de corrélation des trois paramètres de la FHB étaient tous significatifs à $P < 0,0001$ (valeur de 0,36 à 0,63). Une analyse de corrélation supplémentaire révèle que les lignées à deux rangs de haute taille et à maturation tardive ont tendance à être moins affectées par la maladie, la présence de glumes ne semblant pas présenter de corrélation significative avec celle-ci. Plusieurs lignées affichaient une résistance élevée et stable à la FHB similaire à celle des témoins résistants. On pourrait y recourir dans les programmes d'hybridation comme source de résistance ou les homologuer en tant que nouveaux cultivars, si leurs paramètres généraux respectent les normes commerciales.

Mots clés: Désoxynivalénol (DON), dépistage de la brûlure de l'épi causée par Fusarium, grains abîmés par Fusarium, *Fusarium graminearum*, *Hordeum vulgare* L.

Abbreviations: CIMMYT, International Maize and Wheat Improvement Center; DH, days to heading; DON, deoxynivalenol; ELISA, enzyme-linked immunosorbent assay; FDK, Fusarium-damaged kernels; FHB, Fusarium head blight; ICARDA, International Center for Agricultural Research in the Dry Areas; MR, moderately resistant; MS, moderately susceptible; PH, plant height

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Barley (*Hordeum vulgare* L.) is one of the world's major cereal crops, and statistics indicate that Canada accounts for about 6% of world production (FAOSTAT 2012). Most barley (>90%) in Canada is produced in the western provinces, including Alberta, Saskatchewan, and Manitoba, and is used domestically or exported for malt, feed or food (Statistics Canada: <http://www.statcan.gc.ca>, verified 2013 Nov. 27). Outbreaks of Fusarium head blight (FHB) in cereal crops in western Canada were rarely reported before 1990, but their occurrence became more frequent and severe after 1990 with *Fusarium graminearum* Schwabe recognized as the main causal agent of cereal FHB (Clear and Patrick 1990; Clear et al. 1996; Tekauz et al. 2000; Legge et al. 2004; Choo 2006). The increased prevalence of FHB in the 1990s could be ascribed to possible changes in the Fusarium pathogen population, alterations in rainfall patterns, adoption of conservation agriculture practices, and the widespread cultivation of susceptible crop varieties (Tekauz et al. 2000). In contrast to most other diseases on barley, the main concern with FHB is not yield reduction, but the potential accumulation of mycotoxins in the harvested grain, especially deoxynivalenol (DON), which can be toxic to humans and animals. DON contamination of barley grain is also a major concern for the malting and brewing industries, which have set a tolerance limit of 0.5 ppm DON in the grain they use, a standard more stringent than those accepted for human consumption. Use of barley grain contaminated by DON can result in processing problems, including beer off-flavors and gushing, as well as the reality/perception that DON may be a carcinogen (Schwarz et al. 2003; Steffenson et al. 2003).

Host resistance is an important component in FHB management system and has the recognized advantage of being cost-effective and environmentally friendly, although it is hard to achieve due to the lack of known immunity and quantitative inheritance (Capettini et al. 2003). Similar to wheat, Type I (resistance to initial infection) and Type II (resistance to fungal spread within plant tissue) resistance have also been reported in barley, although the former is regarded to be more important (McCallum and Tekauz 2002; Steffenson et al. 2003; Geddes et al. 2008). Point inoculation is used for the evaluation of Type II resistance, while spray inoculation is claimed to be mainly for Type I resistance, although in wheat it is used for a combination of Type I and Type II resistance (Zhu et al. 1999; Steffenson et al. 2003; Choo 2006). In addition, DON content and Fusarium-damaged kernels (FDK) are also used in Canadian barley grading system (Clear et al. 1996; Tekauz et al. 2000), corresponding to Type III and Type IV resistance in wheat, respectively (Mesterhazy et al. 2005). Agronomic and morphological traits have been found to be associated with FHB resistance in barley, which has been elucidated by genetic studies to be derived from either pleiotropic effects or tight linkages (de la Pena et al. 1999; Zhu et al. 1999; Massman et al. 2011). Generally, high stature, late

heading, two-row, lack of laterals, lax and nodding spike, hullless, and lodging resistance are often associated with FHB resistance (Steffenson et al. 2003; Choo 2006).

To identify resistance source for FHB, numerous germplasm screening activities have been carried out in Canada, the United States, China, and Japan, as reviewed by Tekauz et al. (2000), Steffenson et al. (2003), and Choo (2009). Several genotypes with high FHB resistance have been identified and incorporated into varieties, such as Chevron and Peatland from Switzerland, Mimai 114, Zhedar 1, Zhedar 2, CI4196 from China, and Shenmai No. 1 (also known as Gobernadora and Zhenmai 1) from the ICARDA/CIMMYT program in Mexico and Svanhals from Sweden. The weaknesses of these sources were undesirable agronomic attributes, susceptibility to other diseases, and poor grain quality, highlighting the necessity of identification and utilization of new resistance sources from the locally adaptive derivatives of those exotic resistance sources (Legge et al. 2004).

Continuous effort on screening breeding materials for FHB is needed to accumulate genes for FHB resistance in a recurrent-selection approach and to identify new resistance sources. FHB screening at El Batán, Mexico, where CIMMYT headquarters is located, has been conducted under strictly standardized field conditions using artificial inoculation of *F. graminearum* strains, whose aggressiveness and DON chemotypes had previously been identified. Using precision spray inoculation technique coupled with mist irrigation and retention of crop residue from previous years enhances the FHB infection in the nursery (He et al. 2013). The current research was carried out within the long-term research collaborative project between the Field Crop Development Center (FCDC), Alberta, Canada, and the ICARDA Global Barley Enhancement Program in which CIMMYT was participating. The study took place at CIMMYT-Mexico, aiming at the characterization of a set of Canadian breeding materials for FHB resistance, and identification of promising lines with good FHB resistance for further breeding efforts.

MATERIALS AND METHODS

Plant Materials and Field Experiments

A set of 402 advanced breeding lines developed at the FCDC, Alberta, Canada, was used. The lines were derived from bulk F6 head selections, planted as individual head rows in F7, and advanced in subsequent generations for yield trials and disease nurseries. The materials used in this study were at F6:8 or later generation and were thus expected to be homozygous and homogeneous recombinant inbred lines. Five cultivars released in Alberta with known FHB resistance were used as checks, including Seebe (resistant check), AC Metcalfe, CDC Copeland and Xena [moderately resistant (MR) or moderately susceptible (MS) checks], and AC Lacombe (susceptible check).

The experimental field is located at El Batán (altitude of 2240 masl, lat. 19°N, with an average annual precipitation of 625 mm). The plant materials were sown and evaluated for FHB resistance in the summer season (May to September) from 2011 to 2014. In the first 2 yr, lines were planted in Jun. 06 in 1-m double row plots with one replication, whereas in 2013 and 2014 lines were sown in May 14 and May 05 respectively, in a randomized complete block design with two replications. The five checks were randomly distributed in the screening field, with aforementioned replications. The screening nursery was equipped with a programmable misting system with DAN modular microsprinklers spaced at distances of 3 m × 4 m. The system operated automatically from 0900 to 2000 in the first 3 yr and from 1000 to 2200 in 2014, with 10 min of spraying per hour to create a humid environment favourable for FHB development. Barley/maize rotation and conservation agricultural practices were followed in the nursery to enhance natural inoculum.

Field Inoculation and Phenotyping Assays

A mixture of five highly aggressive DON-producing *F. graminearum* isolates was used for field inoculation. The isolates were collected from naturally infected wheat spikes (Mexican *F. graminearum* isolates from wheat and barley were of the same species and chemotype based on our unpublished results) in different locations in Mexico, genotyped with species- and chemotype-specific markers, characterized with rice medium DON assay, and tested for their aggressiveness in greenhouse (He et al. 2013). Conidia of the isolates were equally mixed and adjusted to a concentration of 50 000 (in 2011 and 2012) or 60 000 (in 2013 and 2014) spores mL⁻¹ for field application.

The barley lines were spray inoculated three times at 0, 2, and 4 d after heading (heading was determined when 50% of the spikes fully emerged in a plot), and field FHB investigation was done at 25 days after heading. In 2011 and 2012, FHB incidence (percentage of symptomatic spikes) was scored with a linear scale of 1 through 4, representing incidence levels of less than 25%, 25–50%, 50–75%, and greater than 75%, respectively. **The observations were taken on a whole-plot basis, and only the lines with the score 1 were selected for evaluation in the subsequent year.** In 2013 and 2014, FHB evaluation was based on the 10 spikes of each line (five per row) that had been tagged at heading by red sticky tape. The numbers of total and infected kernels of each spike were recorded for calculating FHB index through the formula:

$$\text{FHB index (\%)} = (\text{Severity} \times \text{Incidence})/100$$

(Stack and McMullen 1994)

with *Severity* for the averaged percentage of diseased kernels and *Incidence* as defined above.

The materials were not tested for FDK and DON in 2011 and 2012, whereas whole plots were harvested at maturity in 2013 and 2014 for the two assays. FDK was estimated by visually checking grain samples in a petri dish, and both symptomatic (pinkish or discoloured) and shriveled grains were scored as FDK and rated on a 0–9 scale. For DON assay, 20-g samples of kernel were ground for each entry, no matter with or without hull (in the cases of hulled and hullless entries, respectively), and a 2-g sub-sample was tested using a Ridascreen® Fast DON ELISA kit (RBiopharm GmbH, Darmstadt, Germany).

Statistical Analyses

The phenotypic data were analyzed by R program ver. 3.0.2. Analysis of variance (ANOVA) was carried out with the *aov* command, and Pearson correlation coefficients were calculated using the *cor.test* function. The data in the ANOVA table were used for calculating heritability estimates, using the formula:

$$h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{g \times y}^2 / y + \sigma_e^2 / ry)$$

in which σ_g^2 stands for genetic variance, $\sigma_{g \times y}^2$ for genotype-by-year interaction, σ_e^2 for error variance, *y* for the number of years, and *r* for the number of replications (Lu et al. 2013). In order to facilitate the identification of stably resistant lines, a composite index was calculated through the sixth root of the product of FHB index, FDK, and DON content in both 2013 and 2014.

RESULTS

FHB incidence was low in 2011, with 324 (80.6%) lines having a score of 1 and only two lines having a score of 3, the highest disease score found in that year. Among the checks, only the susceptible check AC Lacombe had a score of 2 and all the other four had a score of 1. Two-row lines showed better disease resistance than six-row lines, and only 4 two-row lines were found having the score 2 and none having the score 3 (Fig. 1a). In 2012, 324 lines with the score 1 in 2011 were re-evaluated and the disease was better than the previous year, with the proportion of the scores 1 through 4 being 55.9, 32.4, 10.2, and 1.5%, respectively. Again, AC Lacombe showed a score of 2 and other checks of 1. Similar to 2011, six-row lines were more susceptible (Fig. 1b).

In 2013, the 181 most resistant lines in 2012 were planted in two replications for their third year evaluation. There was a wide range of FHB index, from 3.6 to 75.5%, with the resistant check Seebe and the susceptible check AC Lacombe being 7.2 and 47.7%, respectively (Fig. 2a, Table S1). The lines evaluated also showed broad ranges for FDK (0.5–7.0) and DON (0.5–10.2 ppm) (Fig. 2b and c). In 2014, the FHB values were very similar to the previous year, while FDK values were more concentrated on the

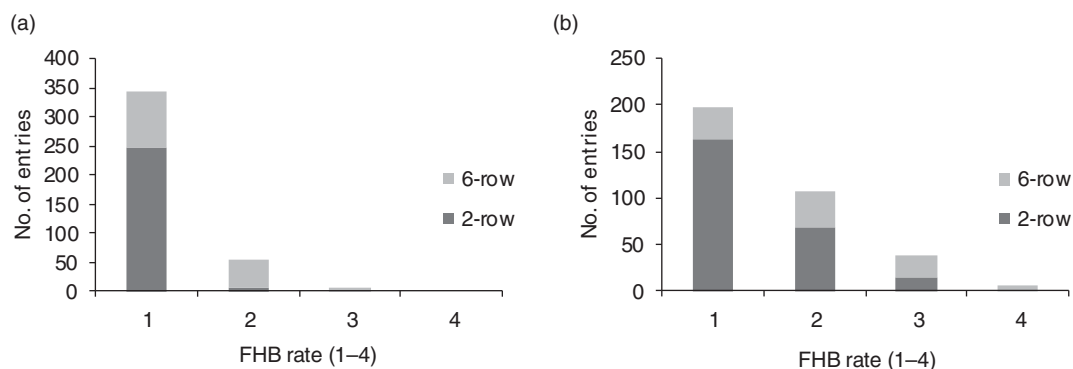


Fig. 1. Distribution of Fusarium head blight incidence in 2011 (a) and 2012 (b).

range of 3–4; but the biggest difference was noticed in DON, with values much higher than those in 2013 (Fig. 2, Table S1).

Genotype effects were significant for all the three FHB related traits, while genotype-by-year effects were significant for FHB index and DON (Table 1). Heritability estimates ranged from high (0.70 for FHB and 0.67 for FDK) to moderate (0.47 for DON). The correlation coefficients among FHB parameters in 2013 and 2014 were all significant at the level of $P < 0.0001$, except the one between DON2013 and FDK2014. Generally, higher correlation coefficients were found for FHB/DON than those for FHB/FDK and FDK/DON (Table 2).

The FHB traits have also exhibited significantly negative correlations with days to heading (DH) and plant height (PH, with the exception of DON2013), while their correlations with row type were positive (Table 3). For hull type, its correlation with FDK was the only one that was significant, showing a trend that hullless lines usually have a lower FDK (Table 3).

Based on the composite index, 21 lines appeared to be more resistant than the resistant check Seebe, e.g. H99069003, J04057002, J02001003. Around 50% lines were better or similar to the three MR/MS checks, although there were still 34 lines that performed worse than the susceptible check (Table S1). The 44 lines

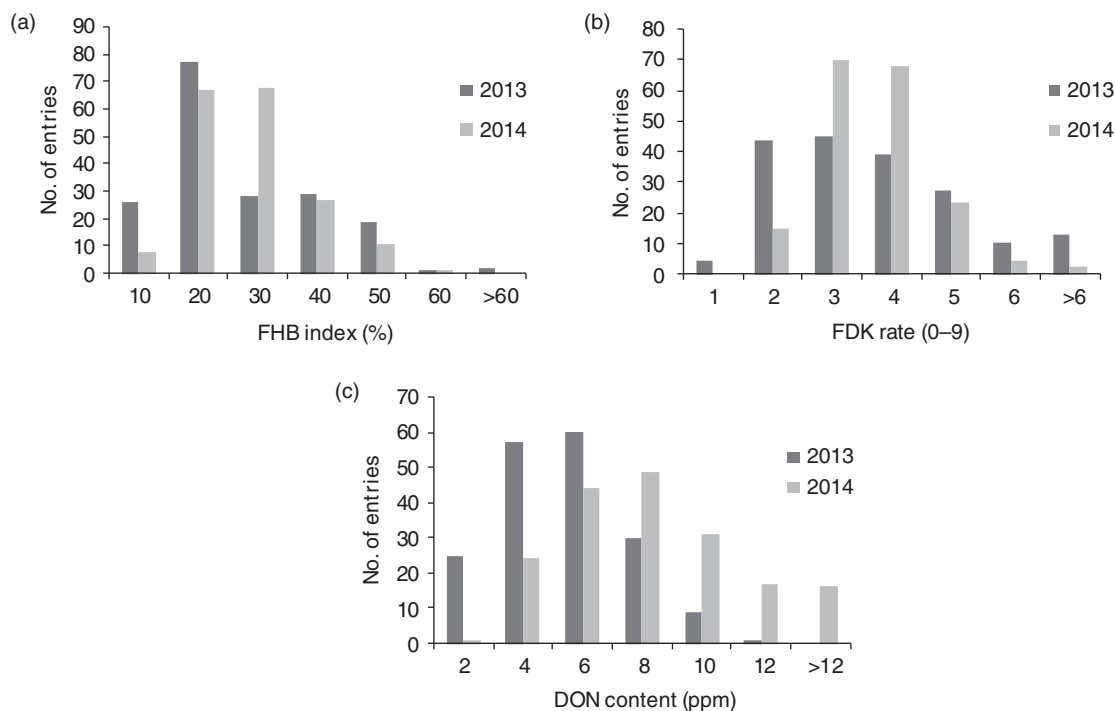


Fig. 2. Distribution of Fusarium head blight (FHB) (a), Fusarium damaged kernels (FDK) (b) and deoxynivalenol content (c) in 2013 and 2014.

Table 1. Analysis of variance for Fusarium head blight index (FHB), Fusarium-damaged kernels (FDK), deoxynivalenol (DON) content, and their heritability estimates over 2013 and 2014

Trait	Source	DF	MS	F value	Pr > F	Heritability
FHB	Genotype	180	396.46	5.42	<0.0001	0.70
	Year	1	206.01	2.81	0.0943	
	G × Y	180	120.72	1.65	<0.0001	
	Rep (Year)	2	174.46	2.38	0.0937	
	Error	358	73.21			
FDK	Genotype	180	4.74	3.27	<0.0001	0.67
	Year	1	0.71	0.49	0.4847	
	G × Y	180	1.55	1.07	0.2946	
	Rep (Year)	2	6.75	4.67	0.0100	
	Error	359	1.45			
DON	Genotype	181	17.13	2.93	<0.0001	0.47
	Year	1	1458.92	249.97	<0.0001	
	G × Y	181	9.14	1.57	0.0002	
	Rep (Year)	2	53.12	9.1	0.0001	
	Error	359	5.84			

with a composite index lower than 5.0 could be regarded as stably resistant lines and be utilized in breeding programs.

DISCUSSION

There was marked difference in disease levels among the 4 yr, with an increasing trend from 2011 to 2014. Three main factors, may have contributed to this, including FHB scoring method, environmental condition, and inoculum concentration. The biggest difference in disease scoring in the first vs. last 2 yr was the adoption of FHB incidence vs. FHB index, and it is noteworthy that the former was based on whole-plots but the latter only on the 10 tagged spikes. In the former case, FHB incidence could be much underestimated due to late-headed spikes, which did not happen in the latter case where only the spikes at heading stage were tagged for subsequent evaluation. Another possible reason on environmental condition is more complicated, involving planting date and precipitation. It has been observed in our FHB screening nursery that early-sown materials usually show heavier FHB than late-sown ones, which was evidenced in 2013 and 2014, when the materials were planted, respectively, 3 and 4 wk earlier than before and higher FHB levels were obtained. Considering the rainfall pattern with a decreasing trend from July to September in those years (El Batán weather station,

CIMMYT), it is tempting to conclude that a high level of precipitation increases the disease, which is true for spawn inoculation, but debatable for spray inoculation. In our case, the reason could be due to the rain-splash facilitated pathogen spread; although spray inoculation was adopted in this experiment, a huge quantity of Fusarium inoculum must be present on the soil surface due to the rotation with maize and conservation agricultural practices, which have been shown to greatly increase FHB epidemics (Champeil et al. 2004). Based on the same assumption, the difference of FHB levels between 2011 and 2012 could be ascribed to the much lower precipitation during the heading period in the former (19.3 mm) than in the latter (75.4 mm). The rainfalls during heading period in 2013 and 2014 were 72.9 and 78.5 mm, respectively, very similar to that in 2012, but a higher inoculum concentration was used, as described above, resulting in a high disease pressure. However, it should be noted that compared with early-headed lines, the rainfall for late lines decreased (50.0 vs. 22.9 mm in 2013 and 49.5 vs. 29.0 mm in 2014). Therefore, late lines with low disease should be utilized with caution, since their “resistance” might actually be disease escape. These results proved the high environmental effects in the determination of resistant germplasm. Different locations and years of testing are necessary to identify resistant genotypes, and the conditions simulated in the nurseries are essential to increase the heritability of the resistance (Capettini et al. 2003).

The negative correlation between FHB and PH has been reported in many studies in wheat, with possible mechanisms of pleiotropy, tight linkage, or disease escape (Buerstmayr et al. 2009). A similar correlation has also been reported in barley studies (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Choo et al. 2004), including the present research, in which PH was significantly negatively correlated with all FHB parameters except DON2013. Nevertheless, it may not be difficult to select short resistant lines, considering the moderate to low correlation coefficients (Table 3).

Similar to other studies, two-row barley lines also exhibited better resistance than six-row lines in our study (Table 3), indicating the challenge in improving FHB resistance for six-row barley. Regarding the correlation of FHB parameters with the hull trait, the

Table 2. Pearson correlation coefficients among Fusarium head blight traits from 2013 and 2014

	FHB2013	FDK2013	DON2013	FHB2014	FDK2014	DON2014
FHB2013	1					
FDK2013	0.54***	1				
DON2013	0.63***	0.37***	1			
FHB2014	0.57***	0.38***	0.31***	1		
FDK2014	0.35***	0.59***	0.17	0.36***	1	
DON2014	0.46***	0.45***	0.32***	0.55***	0.43***	1

*** $P < 0.0001$.

Table 3. Pearson correlation coefficients between FHB parameters and agronomic traits in 2013 and 2014

	DH	PH	Row-type	Hull
FHB2013	−0.60***	−0.34***	0.33***	0.06
FDK2013	−0.32***	−0.49***	0.39***	0.32***
DON2013	−0.55***	−0.11	0.20	−0.07
FHB2014	−0.43***	−0.38***	0.52***	0.04
FDK2014	−0.33***	−0.30***	0.18	0.32***
DON2014	−0.47***	−0.37***	0.42***	0.05

*** $P < 0.0001$.

results were not consistent in literature. According to Clear et al. (1997), over 50% of DON initially present on barley grain could be removed during the dehulling process, which was in agreement with the results of Zhou et al. (1991) and Legzdina and Buerstmayr (2004), in which hulless varieties showed lower DON content than hulled lines. However, Chen et al. (1991) found the opposite trend in a collection of 4163 Chinese barley varieties, and Legge et al. (2004) also noticed the existence of Canadian hulless barley lines with very high DON content. Thus, it seems that the tolerance to DON is highly dependent on genetic background. In the current study, the hull trait did not show correlation with FHB and DON, but it exhibited significant correlation with FDK. This may be caused by an unintentional bias in assessing FDK for hulled vs. hulless lines. For the former, very slight symptoms on glume could be observed and scored as FDK, regardless whether the seeds per se were damaged or not; for the latter, only the real seed damage is taken as FDK. This usually resulted in an overestimation of FDK in hulled lines and an underestimation in hulless lines. Therefore, there may not be significant difference in FDK between hulled and hulless lines used in the current study. To overcome this evaluation problem, it is advisable to score FDK separately for hulled and hulless lines, using their respective resistant and susceptible checks.

Generally, DON content is the most important trait regarding food safety and malting and brewing quality, but it is also the most expensive trait to evaluate compared with FHB index and FDK. Usually, DON content showed the lowest heritability estimates among the three FHB parameters, due to the extra errors introduced during post-harvest processing steps before DON quantification. As reflected in this study, the heritability of DON (0.47) was much lower than those of FHB (0.70) and FDK (0.67), and the replication effects of DON were significant in both years, which did not happen for FHB and happened only at marginal level for FDK (Table 1). Accordingly, the latter two traits were often used in practices to predict DON. In the present study, correlation coefficients of 0.63 and 0.55 were found between FHB and DON in 2013 and 2014, respectively, which were close to the values reported by

Steffenson (1998) of 0.64 and by de la Pena et al. (1999) of 0.75 in the US barley materials, and by Legge et al. (2004) of 0.54–0.73 and by Geddes et al. (2008) of 0.68 in the Canadian varieties. As for the relationship between FDK and DON, previous research indicated lower correlation than that of FHB/DON (Clear et al. 1996; Tekauz et al. 2000), in accordance with our result with correlation coefficients of 0.37 and 0.43 in the 2 yr. The moderate correlations of FHB/DON and FDK/DON justified the screening on FHB and FDK for the identification of varieties with low DON content, but the transformed determination coefficients were generally low (r^2 ranging from 0.19 to 0.40) and there were always outliers, as shown before (Tekauz et al. 2000; Legge et al. 2004) and in our study (Table S1), indicating the importance of performing DON assay for the promising lines with low FHB and FDK.

In the pedigree of the 43 resistant lines with a composite index lower than 5.0, the resistant cultivar Seebe and several MR/MS varieties like I92124, TR232 (syn. AC Metcalfe), and TR238 were frequently observed, indicating known resistance sources. It should be noted that lines with “known resistance” could also be regarded as “new resistance sources” if they are more resistant than their ancestors due to transgressive segregation. For example, J04079153 (pedigree I92124/TR238//SEEBE) is an offspring of Seebe, yet its composite index (3.3) was markedly lower than that of Seebe (4.3), possibly due to the introduction of resistance genes from the two MR/MS lines I92124 and TR238. Several lines for which no known resistant source could be found may represent new sources of resistant. When DH and PH were taken into consideration, lines with both high resistance and good agronomy could be identified. For example the two-row lines H99069003 (pedigree HB335/PHOENIX), T07108001 (CDC COWBOY/CDC RATTAN) and J04076003 (Manley/Leo//TR238//I92124/TR238), and a six-row line H00010004 (H96106/Jaeger) exhibited moderate DH and PH values and disease parameters comparable with the resistant two-row check Seebe (Table S1). The resistant lines identified in this study could be used as new resistance sources or released as cultivars provided they have acceptable resistance to other diseases and good grain quality.

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- Buerstmayr, H., Ban, T. and Anderson, J. A. 2009. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant Breed.* **128**: 1–26.
- Capettini, F., Rasmusson, D. C., Dill-Macky, R., Schiefelbein, E. and Elakkad, A. 2003. Inheritance of resistance to Fusarium head blight in four populations of barley. *Crop Sci.* **43**: 1960–1966.
- Champeil, A., Dore, T. and Fourbet, J. F. 2004. Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by Fusarium in wheat grains. *Plant Sci.* **166**: 1389–1415.
- Chen, X., Yang, Y. and Gao, D. 1991. Preliminary identification of sources resistant to scab in Chinese barley germplasm. *J. Zhejiang Agric. Sci.* **2**: 91–97.
- Choo, T. M. 2006. Breeding barley for resistance to Fusarium head blight and mycotoxin accumulation. *Plant Breed. Rev.* **26**: 125–169.
- Choo, T. M. 2009. Fusarium head blight of barley in China. *Can. J. Plant Pathol.* **31**: 3–15.
- Choo, T. M., Vigier, B., Shen, Q. Q., Martin, R. A., Ho, K. M. and Savard, M. 2004. Barley traits associated with resistance to Fusarium head blight and deoxynivalenol accumulation. *Phytopathology* **94**: 1145–1150.
- Clear, R. M. and Patrick, S. K. 1990. *Fusarium* species isolated from wheat samples containing tombstone (scab) kernels from Ontario, Manitoba, and Saskatchewan. *Can. J. Plant Sci.* **70**: 1057–1069.
- Clear, R. M., Patrick, S. K., Nowicki, T., Gaba, D., Edney, M. and Babb, J. C. 1997. The effect of hull removal and pearling on *Fusarium* species and trichothecenes in hulless barley. *Can. J. Plant Sci.* **77**: 161–166.
- Clear, R. M., Patrick, S. K., Platford, R. G. and Desjardins, M. 1996. Occurrence and distribution of *Fusarium* species in barley and oat seed from Manitoba in 1993 and 1994. *Can. J. Plant Pathol.* **18**: 409–414.
- de la Pena, R. C., Smith, K. P., Capettini, F., Muehlbauer, G. J., Gallo-Meagher, M., Dill-Macky, R., Somers, D. A. and Rasmusson, D. C. 1999. Quantitative trait loci associated with resistance to Fusarium head blight and kernel discoloration in barley. *Theor. Appl. Genet.* **99**: 561–569.
- Geddes, J., Eudes, F., Tucker, J. R., Legge, W. G. and Selinger, L. B. 2008. Evaluation of inoculation methods on infection and deoxynivalenol production by *Fusarium graminearum* on barley. *Can. J. Plant Pathol.* **30**: 66–73.
- He, X., Singh, P. K., Duveiller, E., Schlang, N., Dreisigacker, S. and Singh, R. P. 2013. Identification and characterization of international Fusarium head blight screening nurseries of wheat at CIMMYT, Mexico. *Eur. J. Plant Pathol.* **136**: 123–134.
- Legge, W. G., Therrien, M. C., Tucker, J. R., Banik, M., Tekauz, A., Somers, O., Savard, M. E., Rossnagel, B. C., Lefol, E., Voth, D. and others 2004. Progress in breeding for resistance to Fusarium head blight in barley. *Can. J. Plant Pathol.* **26**: 436–442.
- Legzdina, L. and Buerstmayr, H. 2004. Comparison of infection with Fusarium head blight and accumulation of mycotoxins in grain of hulless and covered barley. *J. Cereal Sci.* **40**: 61–67.
- Lu, Q., Lillemo, M., Skinnies, H., He, X., Shi, J., Ji, F., Dong, Y. and Bjornstad, A. 2013. Anther extrusion and plant height are associated with Type I resistance to Fusarium head blight in bread wheat line ‘Shanghai-3/Catbird’. *Theor. Appl. Genet.* **126**: 317–334.
- Ma, Z., Steffenson, B. J., Prom, L. K. and Lapitan, N. L. 2000. Mapping of quantitative trait loci for Fusarium head blight resistance in barley. *Phytopathology* **90**: 1079–1088.
- Massman, J., Cooper, B., Horsley, R., Neate, S., Dill-Macky, R., Chao, S., Dong, Y., Schwarz, P., Muehlbauer, G. J. and Smith, K. P. 2011. Genome-wide association mapping of Fusarium head blight resistance in contemporary barley breeding germplasm. *Mol. Breed.* **27**: 439–454.
- McCallum, B. and Tekauz, A. 2002. Influence of inoculation method and growth stage on Fusarium head blight in barley. *Can. J. Plant Pathol.* **24**: 77–80.
- Mesterhazy, A., Bartok, T., Kaszonyi, G., Varga, M., Toth, B. and Varga, J. 2005. Common resistance to different Fusarium spp. causing Fusarium head blight in wheat. *Eur. J. Plant Pathol.* **112**: 267–281.
- Schwarz, P., Leonard, K. and Bushnell, W. 2003. Impact of Fusarium head blight on malting and brewing quality of barley. Pages 395–419 in K. Leonard and W. Bushnell, eds. *Fusarium head blight of wheat and barley*. American Phytopathological Society, St. Paul, MN.
- Stack, R. W. and McMullen, M. P. 1994. A visual scale to estimate severity of Fusarium head blight in wheat. North Dakota State University Extension Service. 1095 pp.
- Steffenson, B., Leonard, K. and Bushnell, W. 2003. Fusarium head blight of barley: impact, epidemics, management, and strategies for identifying and utilizing genetic resistance. Pages 241–295 in K. Leonard and W. Bushnell, eds. *Fusarium head blight of wheat and barley*. American Phytopathological Society, St. Paul, MN.
- Steffenson, B. J. 1998. Fusarium head blight of barley: epidemics, impact, and breeding for resistance. *Technical quarterly – Master Brewers Association of the Americas* **35**: 177–184.
- Tekauz, A., McCallum, B. and Gilbert, J. 2000. Review: Fusarium head blight of barley in western Canada. *Can. J. Plant Pathol.* **22**: 9–16.
- Zhou, X., Cao, M. and Liang, X. 1991. Screening and testing of barley varieties for scab resistance. *Acta Phytopythologica Sin.* **18**: 261–265.
- Zhu, H., Gilchrist, L., Hayes, P., Kleinhofs, A., Kudrna, D., Liu, Z., Prom, L., Steffenson, B., Toojinda, T. and Vivar, H. 1999. Does function follow form? Principal QTLs for Fusarium head blight (FHB) resistance are coincident with QTLs for inflorescence traits and plant height in a doubled-haploid population of barley. *Theor. Appl. Genet.* **99**: 1221–1232.